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TITLE: Elucidating the Mechanism of p27 Inactivation by the Bcr-Abl Tyrosine Kinase

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14. ABSTRACT Inhibition of Bcr-Abl kinase activity in Mo7e – P210BCR-ABL cells induces accumulation of cells in G0/1. This is associated with (a) increased total levels of p27, (b) accumulation of p27 in the nucleus but not the cytoplasm, and (c) decrease of nuclear but not cytoplasmic Cdk2/cylin E kinase activity. Despite the decrease of Cdk2/cyclin E activity, most of the nuclear p27 is phosphorylated on threonine 187, consistent with diminished nuclear degradation. In accordance with this, the expression of KPC (subunit 1 and 2), the ubiquitin ligase responsible for targeting cytoplasmic p27 for degradation, was not affected by inhibition of Bcr-Abl. The reduced degradation of nuclear p27 is likely due to downregulation of Skp2, the F-box protein of the SCFSKP2 complex that specifically recognizes T187 phosphorylated p27 and targets it for proteasomal degradation. Although no major differences in lineage marker expression were seen in Skp2 knockout mice compared to wildtype mice, there appears to have reduced clonogenicity in response to growth factors and upon expression of Bcr-Abl. This may lead to reduced leukomogenicity of Bcr-Abl in a Skp2 -/- background.					
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## INTRODUCTION

Bcr-Abl is a constitutively active tyrosine kinase that is responsible for the pathogenesis of chronic myeloid leukemia (CML) and some cases of acute lymphoblastic leukemia (ALL)(1). Imatinib, a specific inhibitor of Bcr-Abl is effective in patients with CML and Bcr-Abl-positive ALL, however relapse after an initial response is common in patients with advanced disease(2,3). Therefore new therapeutic approaches are required. p27 is an inhibitor of cyclin dependent kinases but has additional functions. It directly inhibits the activity of cdk2/cyclin E and cdk2/cyclin A complexes but it also serves as an assembly factor to generate these complexes(4-6). p27 regulation is complex, involving transcriptional, posttranslational as well as topological levels of control. In Bcr-Abl expressing cell lines p27 is downregulated, and re-expression from ectopic vectors induces cell cycle arrest(7,8). In primary CML cells there is mislocalization to the cytoplasm, where the protein is unable to exert its inhibitory function towards cdk2(9). The precise mechanisms responsible for the deranged regulation of p27 in Bcr-Abl expressing cells are unknown. The nuclear/cytoplasmic distribution of p27 is regulated by shuttle proteins as well as phosphorylation on specific sites, including serine 10(10) and threonine 157 (in humans)(11,12). Proteasomal degradation occurs via distinct nuclear and cytoplasmic pathways. The key ubiquitin ligase responsible for nuclear degradation is the SCF<sup>Skp2</sup> complex, in which Skp2 plays the key role of the F-box protein recognizing p27 after the latter has been phosphorylated on T187 by cyclin E/cdk2(13,14). Cytoplasmic proteasomal degradation occurs via a novel ubiquitin ligases termed KPC(15).

In many solid tumors cytoplasmic mislocalization is observed at the adenoma stage while more advanced tumors are characterized by down-regulation of overall protein levels. Low levels of p27 tend to correlate with a poor prognosis, and there is an inverse relationship between Skp2 and p27 levels, suggesting that Skp2 may be responsible for the low levels of p27(16,17). In CML, the mechanisms underlying downregulation and mislocalization of p27 are not well understood; however the fact that low p27 levels have adverse prognostic significance in solid tumors suggests that reversing these abnormalities may be therapeutically beneficial. Based on these considerations 2 specific aims were formulated:

**Specific aim 1.** To determine, whether Bcr-Abl regulates the activity of the SCFSKP complex, and whether Skp2 is required for induction of leukemia in a murine model of CML.

**Specific aim 2.** To determine the cellular localization of p27, whether it is associated with phosphorylation of specific residues and whether it influences the degradation of the protein.

## BODY

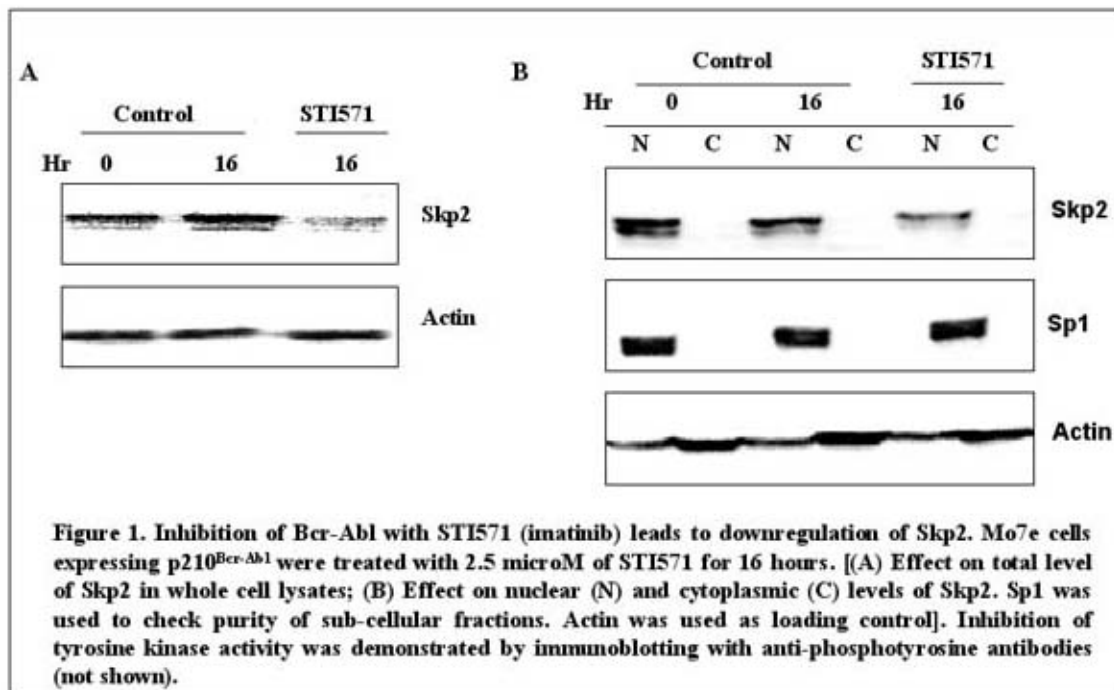
*Task 1.* To determine the ubiquitination activity toward p27 in Mo7e leukemia cells expressing the Bcr-Abl oncoprotein

- a. Generate a GST-p27 construct and test ubiquitination capacity toward recombinant p27 in cytoplasmic and nuclear extracts from Mo7-p210<sup>Bcr-Abl</sup> cells (treated and untreated with STI571 [imatinib]). (Months 1-2)
- b. Optional follow-up studies: Determine the association of p27 with cyclin D3 and Grb-3-3 by co-immunoprecipitation. (Month 3)

We have generated FLAG-tagged p27 constructs for ubiquitination assays, including wild type p27 as well as S10A, T157A, and T187A mutants. Due to the fact that Dr. Lanker has left OHSU, the technical expertise to perform ubiquitination assays of p27 in nuclear and cytoplasmic lysates is not longer available on site. We have therefore started to set up these assays in our laboratory but have not yet been able to generate consistent results. In addition, with the data shown below it is very likely that cytoplasmic p27 stability is not regulated by Bcr-Abl kinase activity, rendering the ubiquitination experiments a lesser priority.

**Task 2.** To determine the expression of Skp2 and possibly other SCF<sup>SKP2</sup> (such as Cks1) in a panel of BCR-ABL-positive leukemia cell lines. (Month 3, in parallel with task 1b)

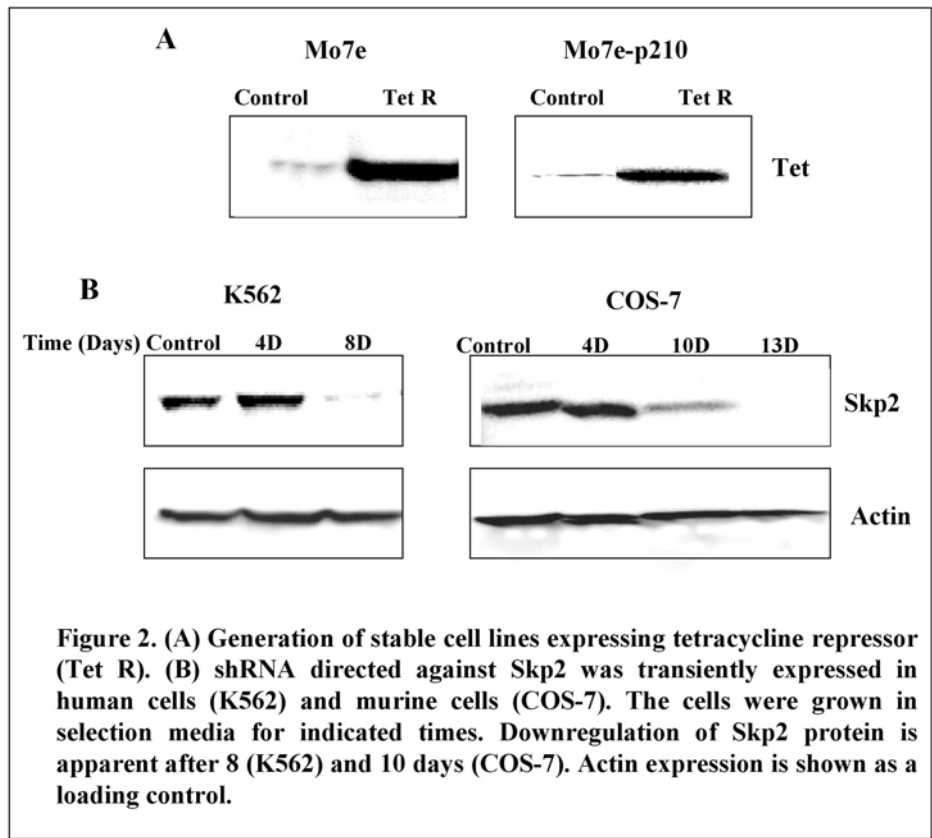
We tested several Bcr-Abl expressing cell lines, including Mo7e – p210<sup>BCR-ABL</sup>, K562 and BaF3-p210<sup>BCR-ABL</sup> cells for expression of Skp2. We find that Skp2 is consistently expressed in BCR-ABL-positive cell lines and that the level of expression decreases with inhibition of Bcr-Abl kinase activity (Figure 1). This is consistent with the notion that Skp2 expression is regulated by Bcr-Abl kinase activity and identifies Skp2 as a downstream target of Bcr-Abl. In order to examine whether these effects were specific to Bcr-Abl expressing cells or were a general phenomenon associated with the expression of an oncogenic tyrosine kinase we have also started to investigate cell lines transformed by different tyrosine kinases, including BaF/3 cells expressing D816V mutant Kit and human lines expressing FLT3-ITD and V617F mutant Jak2. Thus far, results have not been conclusive.



**Task 3.** To determine, if ectopic Skp2 expression rescues p27 expression and cell cycle progression in Mo7-p210<sup>BCR-ABL</sup> cell treated with imatinib. (Month 4)

Several considerations led us to change strategy. (a) One of the major translational questions to be answered in this project is whether Skp2 may be a therapeutic target – for example, it may be possible to develop specific small molecule inhibitors that interfere with the recognition of p27 by Skp2. This in mind, we realized that it would be more critical to devise a system that permits assessing Skp2's relevance in a direct manner rather than by the experiments originally planned, which would have been based on over-expressing the protein while endogenous protein is downregulated by inhibition of Bcr-Abl. (b) The induction of apoptosis along with the G1/0 arrest in Mo7-p210<sup>BCR-ABL</sup> cells treated with imatinib limits the time frame of the experiments to maximal 20 hours, at which time the cells apoptose. We therefore decided to down-regulate Skp2 expression using shRNA using a lentivirus-based inducible system. The additional advantage of this system is that it can be used in primary cells as the integration of the shRNA construct is stable. As the system is inducible, the expected effect of inhibiting Skp2 expression, namely up-regulation of p27 with subsequent cell cycle arrest can be avoided. Until now we have generated Mo7e and Mo7-p210<sup>BCR-ABL</sup> cells expressing the tetracycline responsive

transcriptional regulator (Figure 2A), and we were able to show down-regulation of Skp2 in K562 and COS-7 cells using Skp2 shRNA (Figure 2B).



**Task 4.** To test the requirement of Skp2 for leukemia induction by Bcr-Abl.

- Determine the capacity of *BCR-ABL* to transform Skp2 <sup>-/-</sup> murine embryonal fibroblasts compared to wildtype fibroblasts and B cells (Months 5-6)
- Determine the capacity of *BCR-ABL* to induce a myeloproliferative syndrome in Skp2 <sup>-/-</sup> mice compared to wildtype mice (Month 5-9, partially in parallel with task 4a)

We have focused on the question whether Bcr-Abl is capable of transforming B-cells and induce a myeloproliferative disease in Skp <sup>-/-</sup> compared to Skp2 <sup>+/+</sup> mice. Unexpectedly and in contrast to published data we find that Skp2 <sup>-/-</sup> mice are born at much lower than Mendelian frequency (Table 1). In addition most Skp <sup>-/-</sup> mice do not breed, further limiting the availability of KO mice for experimentation. In initial experiments we determined whether Skp2 <sup>-/-</sup> bone marrow cells exhibit a major defect in the distribution of immunophenotypically defined subsets of cells (Table 2).

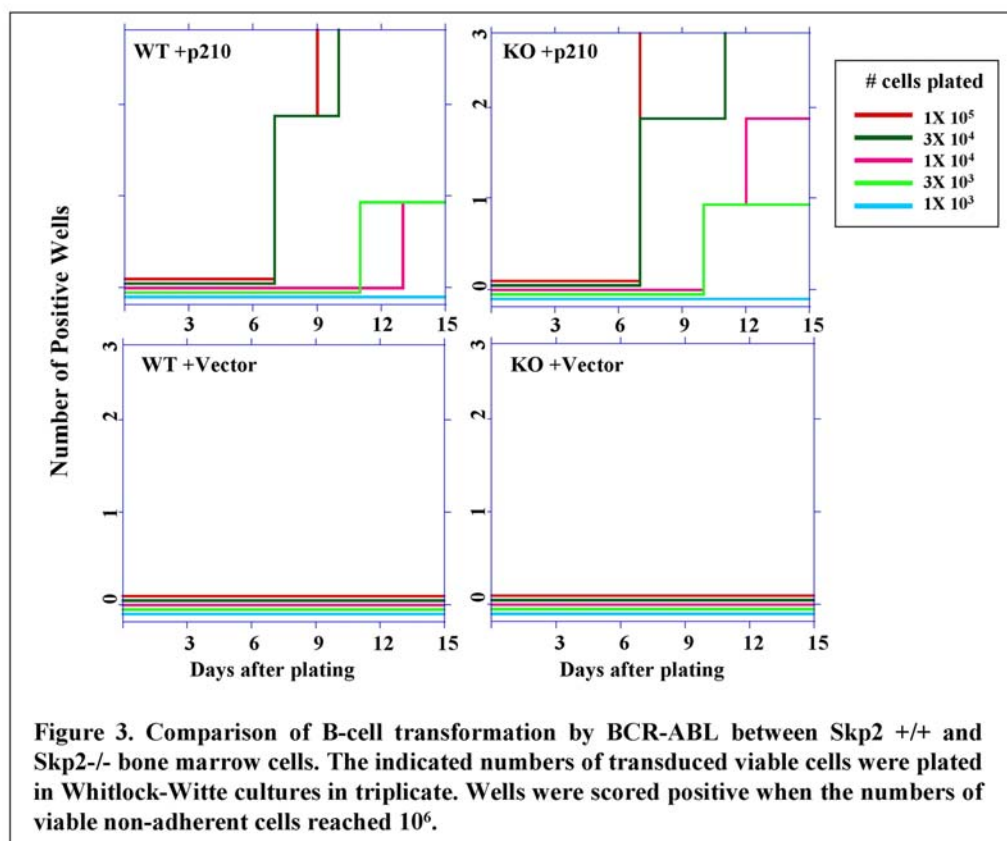
Litter Group	Parents		Number of pups born	Pups Genotype		
	Father	Mother		WT	KO	HET
1.	HET	HET	7	2	-	5
2.	HET	HET	4	2	-	2
3.	HET	HET	7	3	1	3
4.	HET	HET	6	-	-	6
5.	HET	HET	4	2	-	2

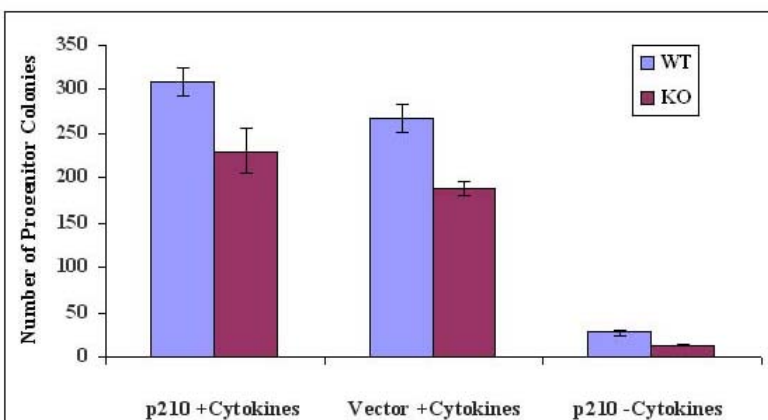
**Table 1.** Distribution of genotypes in 5 litters using Skp2 <sup>+/+</sup> mice. A total 28 mice were born from the 5 heterozygote-heterozygote breedings, but only 1 rather were <sup>-/-</sup> compared to 7 mice as expected by mendelian frequency.

Marker	Skp2 <sup>+/+</sup>	Skp2 <sup>-/-</sup>
Erythroid	4.9%	20.0%
Gr-1	90.2%	86.4%
CD11b	92.1%	85.9%
B220	52.4%	49.2%
Thy12	43.2%	45.5%
CD 133	18.0%	21.1%
CD 45	91.5%	97.6%
CD34	9.1%	13.4%
Thy11	8.3%	5.3%
Sca-1	10.9%	13.1 %
CD117	5.1%	4.5%

**Table 2. Immunophenotyping of bone marrow cells derived from Skp2<sup>+/+</sup> and Skp2<sup>-/-</sup> mice. No consistent differences in the expression of a panel of lineage specific and early progenitor cell markers were observed**

No consistent differences were observed, suggesting that any difference vs. wildtype would be primarily attributable to the role of Skp2 in Bcr-Abl-induced leukemogenesis rather than to differences in the number of target cells available for transformation. In a first set of experiments we compared B-cell transformation and cytokine-independent myeloid colony formation in Skp<sup>-/-</sup> vs. Skp2<sup>+/+</sup> bone marrow cells transduced with Bcr-Abl. We find no consistent difference in B-cell transformation (Figure 3), while the formation of myeloid colonies in the absence of cytokines was slightly reduced in Skp2<sup>-/-</sup> bone marrow (Figure 4). Similarly, myeloid colony formation in response to cytokines was also reduced in Skp2<sup>-/-</sup> marrow. Given the low numbers of Skp<sup>-/-</sup> mice, only one bone marrow transplantation experiment could be carried out thus far (Table 3). Since due to unknown reasons the viability of cells prior to injection into the recipients was very low in this experiment we do not consider these data to be reliable. After intense efforts we have now 4 knockout animals available (Table 4). This will allow for transplantation of BCR-ABL transduced bone marrow cells into 8 recipients.





**Figure 4.** Comparison of myeloid colony formation between *Skp2*  $+/+$  and *Skp2*  $-/-$  bone marrow cells. Bone marrow cells were transduced with BCR-ABL or empty vector and plated in methylcellulose in the presence and absence of cytokines. The histograms show the average numbers of colonies from triplicate assays. No colonies were recovered from cells transduced with empty vector and grown in the absence of cytokines (not shown).

Sex	HET	WT	KO
Male	22	11	2
Female	12	7	2

**Table 4.** Size of current *Skp2* mouse colony

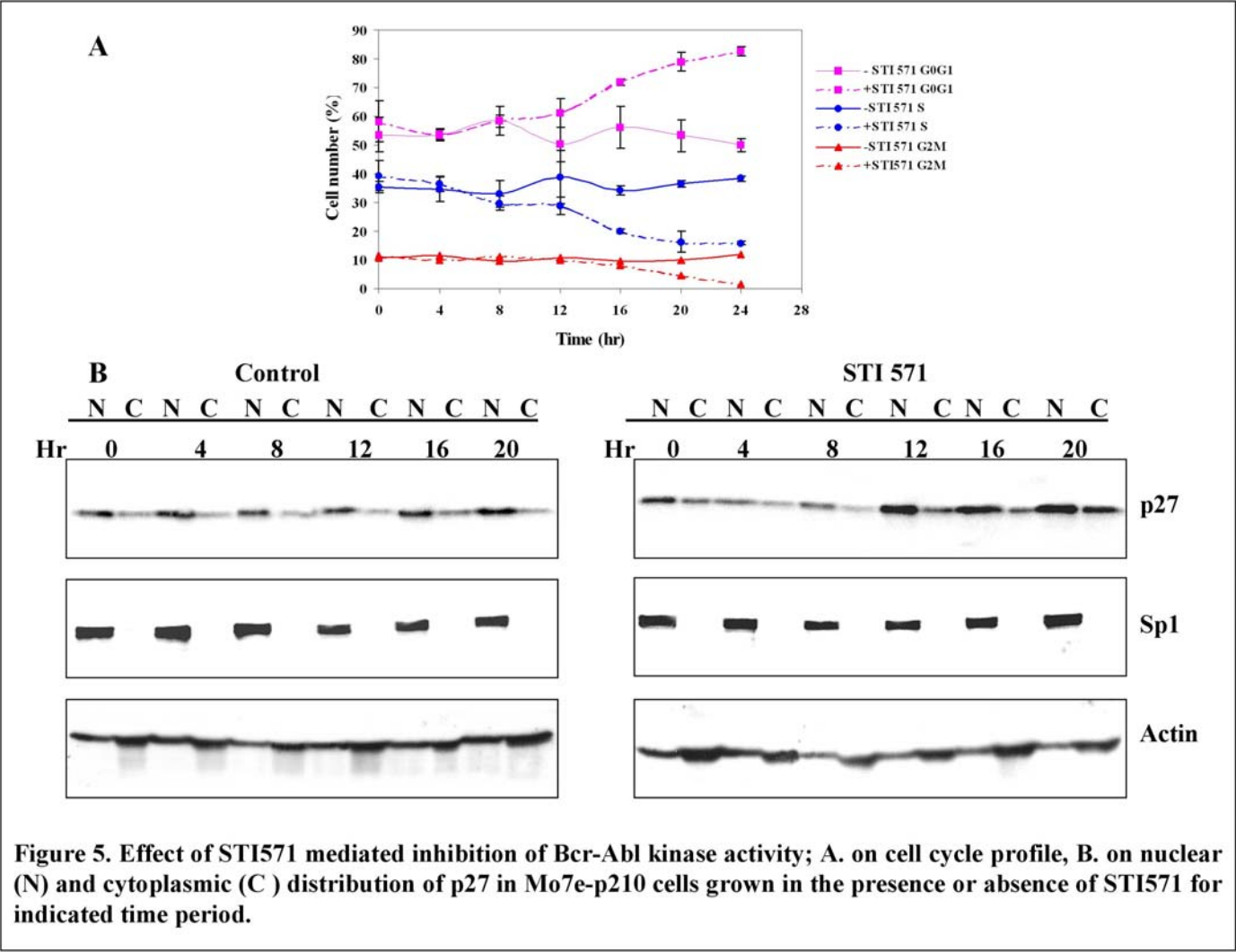
Blood counts 13 days after bone marrow transplantation								
Experimental Group	WBC (10e3/mm3)	RBC (10e6/mm3)	HGB (g/dl)	HCT (%)	PLT (10e3/mm3)	LYM (%)	MON (%)	GRA (%)
WT+Bcr-Abl* (n=3)	18.0 $\pm$ 2.6	12.6 $\pm$ 0.3	20.0 $\pm$ 0.7	64.3 $\pm$ 1.9	387.3 $\pm$ 85.4	28.3 $\pm$ 4.5	8.6 $\pm$ 0.7	63.1 $\pm$ 3.8
WT control (n=4)	4.7 $\pm$ 2.1	9.7 $\pm$ 0.9	15.7 $\pm$ 1.3	49.7 $\pm$ 4.3	457.0 $\pm$ 19	26.4 $\pm$ 6.1	11.7 $\pm$ 2.1	61.7 $\pm$ 7.5
KO+Bcr-Abl (n=4)	1.5 $\pm$ 0.12	8.3 $\pm$ 0.5	13.8 $\pm$ 0.7	43.0 $\pm$ 1.68	107.0 $\pm$ 49.7	-	-	-
KO control** (n=3)	0.6 $\pm$ 0.2	4.0 $\pm$ 0.7	6.2 $\pm$ 1.1	17.2 $\pm$ 2.7	30.0 $\pm$ 11.1	-	-	-
Blood counts 20 days after bone marrow transplantation								
WT+Bcr-Abl* (n=1)	32.4	14.6	22.4	74.0	704.0	26.0	5.7	68.3
WT control (n=4)	4.3 $\pm$ 2.3	10.4 $\pm$ 2.6	16.9 $\pm$ 4.1	46.4 $\pm$ 1.3	492.5 $\pm$ 173.5	51.3 $\pm$ 4.2	10.9 $\pm$ 0.8	37.7 $\pm$ 3.9
KO+Bcr-Abl (n=4)	3.1 $\pm$ 1.2	7.4 $\pm$ 1.5	12.8 $\pm$ 2.2	39.12 $\pm$ 5.9	355.0 $\pm$ 335.7	40.2 $\pm$ 3.2	11.6 $\pm$ 0.8	48.2 $\pm$ 2.6
KO control** (n=3)	Sacrificed after 13 days due to poor health condition							

**Table 3.** Comparison of Bcr-Abl-induced leukemia in *Skp2*  $+/+$  vs. *Skp2*  $-/-$  mice. Bone marrow cells were transduced with a BCR-ABL retrovirus or the empty construct and injected into lethally irradiated recipients. Blood counts are shown on day 13 and 20 after transplant. \* Of the *Skp2*  $+/+$  mice transplanted with BCR-ABL-transduced marrow, one died on day 13, other three died on day 17, 20 and 22. \*\* Of the *Skp2*  $-/-$  mice transplanted with empty construct-transduced marrow, one died on day 13. Due to low viability of the cells after retroviral transduction, we consider the data derived from this experiment unreliable, although they may suggest the leukemia induction is delayed in *Skp2*  $-/-$  mice.



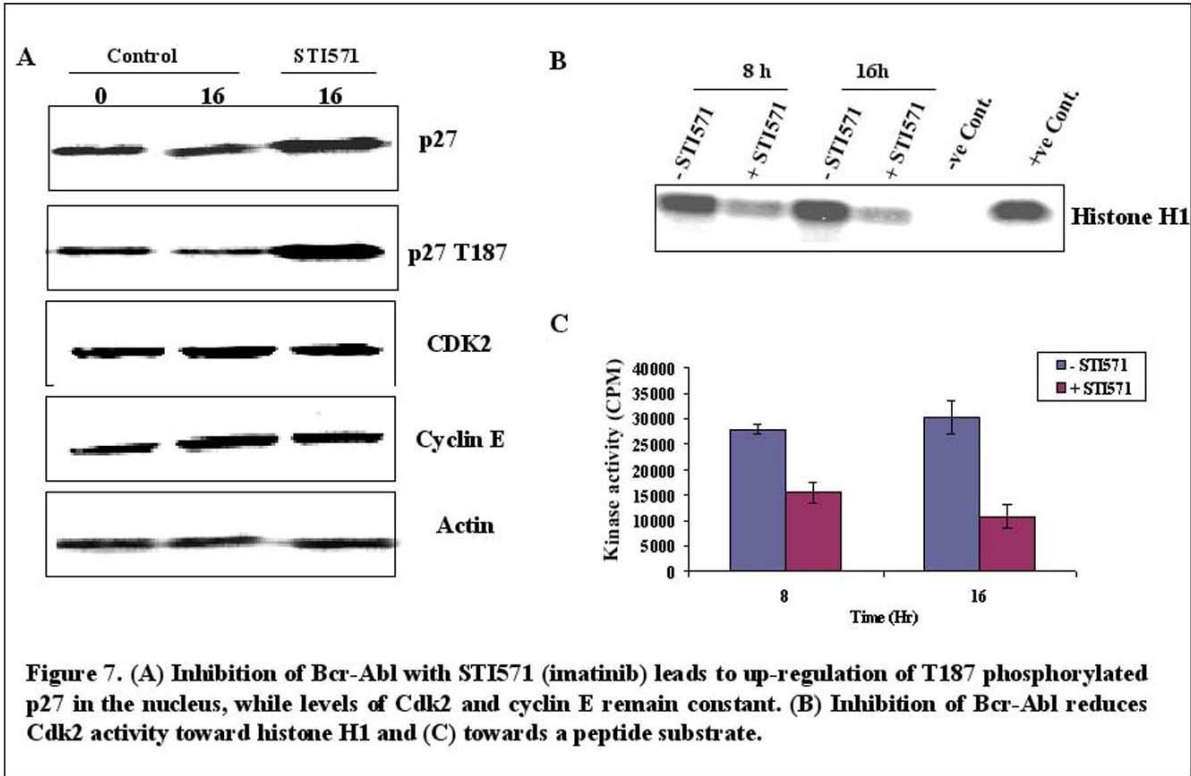
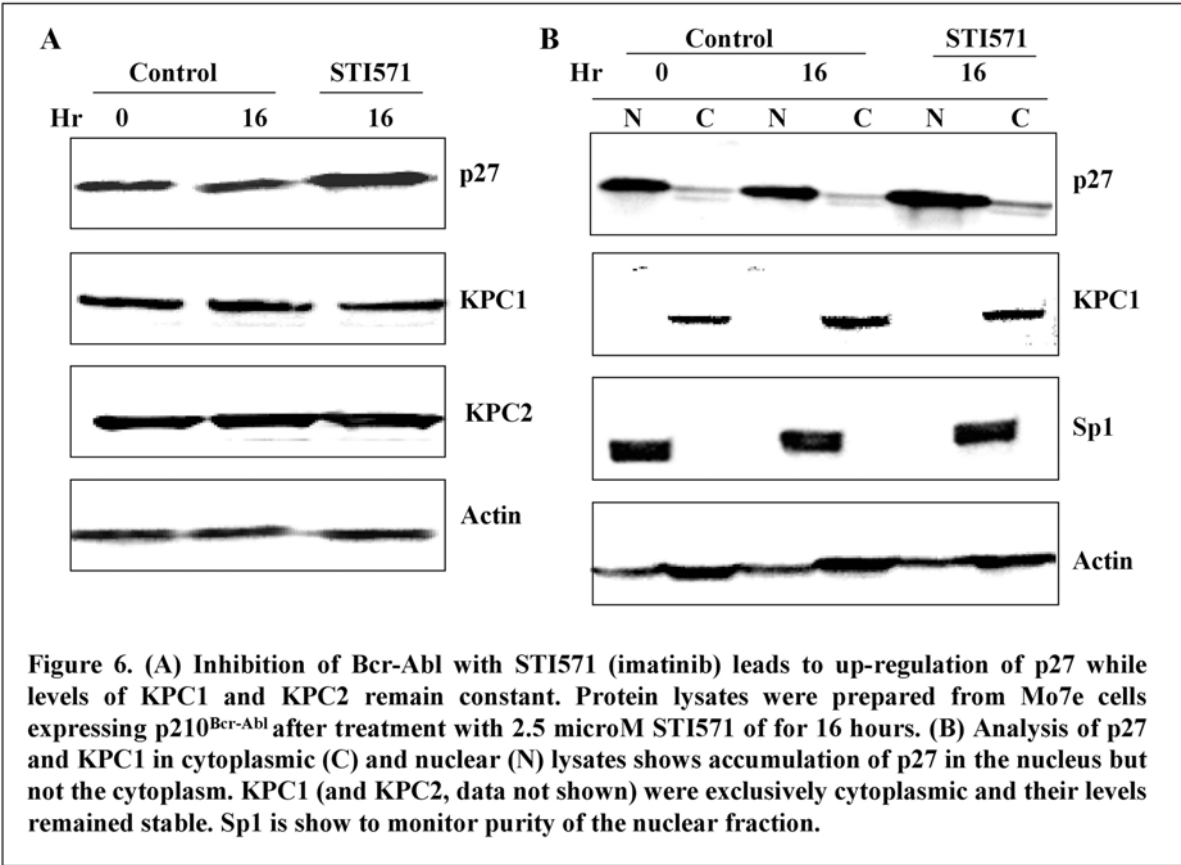
- Task 5. To determine the intracellular localization, stability and phosphorylation of p27 in Mo7-p210<sup>BCR-ABL</sup> treated and untreated with imatinib.
- Determine the p27 expression and protein turnover (pulse chase) in cytoplasmic and nuclear lysates (Month 10)
  - Optional follow-up studies (association of p27 with cyclin D, etc.) (Month 11)
  - Studies using leptomycin B to inhibit nuclear export of p27 (Month 11)
  - Determine the phosphorylation of Thr-157 and Ser-10 of p27 and its dependence on Akt and/or hKIS (Months 11-12)

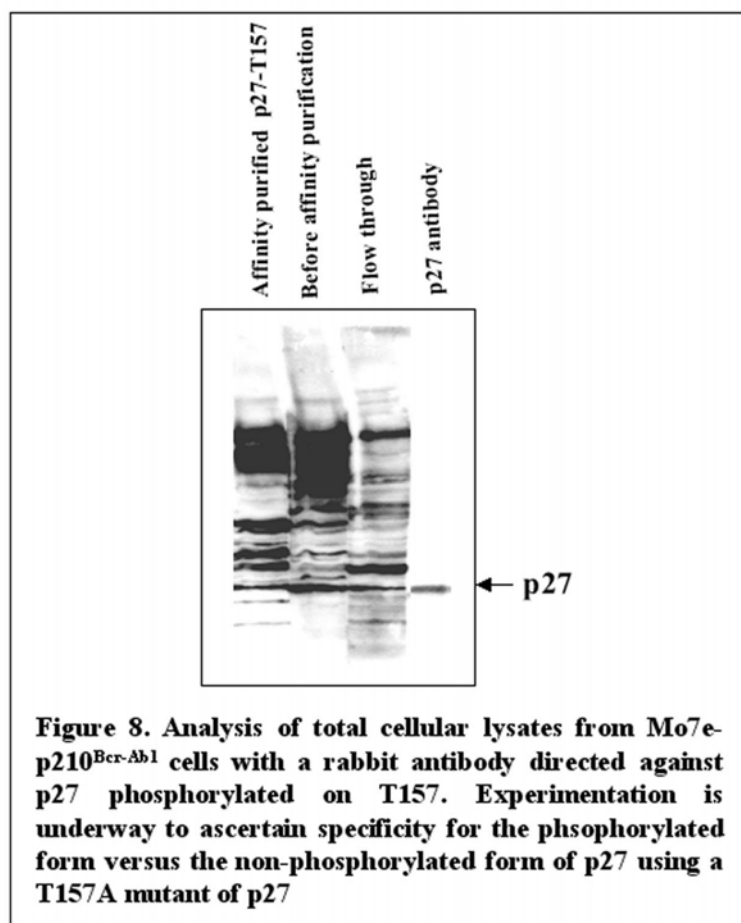
We have determined the time course of p27 upregulation and cell cycle arrest in Mo7-p210<sup>BCR-ABL</sup> cells upon inhibition of Bcr-Abl kinase activity (Figure 5). We clearly demonstrate that the upregulation of p27 precedes the accumulation of cells in G0/1, suggesting that it is the primary event. Analysis of nuclear and cytoplasmic lysates indicated that the accumulation is almost exclusively the result of increased nuclear levels, while cytoplasmic levels remain practically constant (Figure 5).



While this work was in progress, Kamura et al(15) reported on the identification of KPC, a novel ubiquitin ligase that targets cytoplasmic p27 for degradation. We obtained antibodies against the two components of the KPC complex (KPC1 and KPC2) and demonstrated that levels are not regulated by Bcr-Abl, in agreement with the stable cytoplasmic p27 levels. This corroborates the notion that in the Mo7-p210<sup>BCR-ABL</sup> cells cytoplasmic p27 is not regulated by Bcr-Abl (Figure 6). In contrast we found nuclear p27 levels strongly increase upon

inhibition of Bcr-Abl and that most of this p27 is phosphorylated on T187, despite the fact that cyclinE/cdk2 activity is decreased (Figure 7). These data concur with the data on Skp2 down-regulation and suggest that Skp2 is the critical mediator of p27 degradation in Bcr-Abl-positive cells.



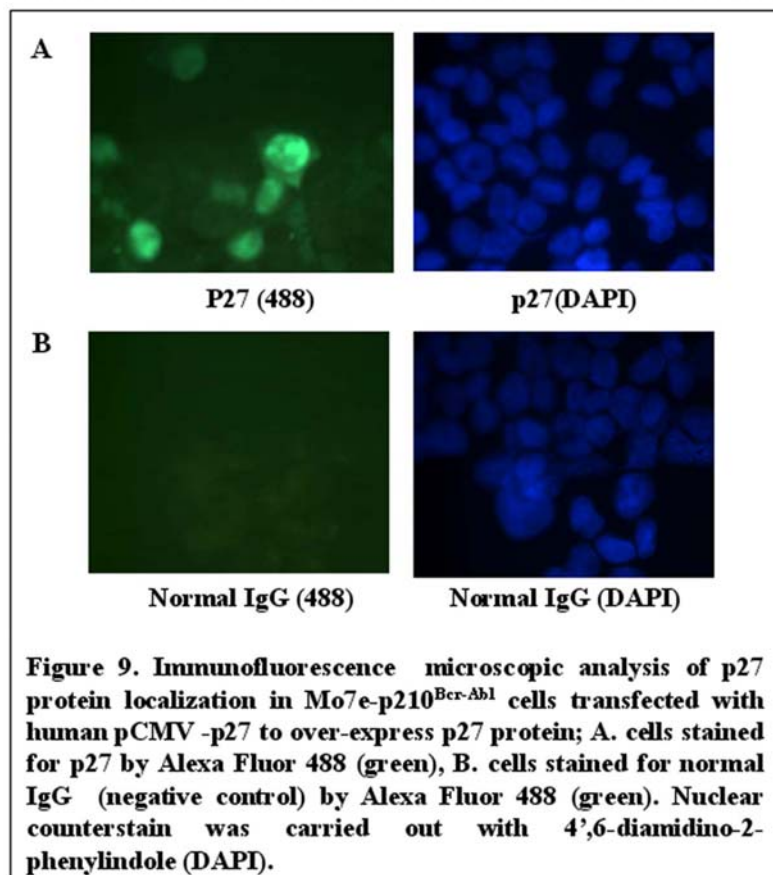


We were unable to show any differences in S10 p27 phosphorylation, although these experiments were hampered by the poor quality of available antibodies. It remains possible that phosphorylation of T157 is also relevant, as the phosphorylation of this residue by a PI3 kinase dependent pathway has been shown to lead to cytoplasmic relocation of p27, and the inhibition of this phosphorylation could also lead to nuclear retention of the protein. As cytoplasmic levels remained constant this is less likely. Nonetheless, we have generated an anti phospho - T157 antibody that is currently undergoing quality testing (Figure 8). As mentioned above we have generated a Flag-tagged T157A mutant of p27 and plan to use this construct to test the specificity of this antibody. If specific for phosphorylated T157, this reagent will allow us to clarify this question. We have also established and optimized immuno-cytochemistry for p27 to help in analyzing intracellular distribution (Figure 9).

### Personnel

The following personnel worked on and received commensurate salary support from this grant:

- Michael Deininger, MD PhD – Principal Investigator
- Paul Yoshihara, PhD – Senior Research Associate
- Taiping Jia – Research Assistant
- Stephanie Willis –Research Associate



## KEY RESEARCH ACCOMPLISHMENTS

- Identification of Skp2 as the most likely critical downstream target of Bcr-Abl kinase activity that mediates degradation of p27
- Identification of the nuclear p27 pool as the Bcr-Abl target
- Preliminary results suggesting that myeloid transformation may be delayed in Skp -/- cells

## REPORTABLE OUTCOMES

- A manuscript summarizing the main findings is in preparation and will be completed as soon as the bone marrow transplant experiments have been carried out.

## CONCLUSIONS

Using this hypothesis development award, we have:

- Identified Skp2 as the most likely candidate mediating the downregulation of p27 by the Bcr-Abl tyrosine kinase. If confirmed in subsequent experiments, our results would imply Skp2 as a therapeutic target in BCR-ABL-positive leukemia;
- Generated a number of reagents that will be invaluable in further and more precisely addressing the question of p27 regulation in BCR-ABL-positive cells, including primary cells from patients with CML and BCR-ABL-positive ALL.

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## APPENDIX

- CV

### MICHAEL WERNER NIKOLAUS DEININGER

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#### EDUCATION

Primary School: Schwarzenbach (Germany)	1969-73
Secondary School: Jean-Paul-Gymnasium Hof (Germany)	1973-82
Best final exam in the state of Bavaria (867/900 possible points)	

#### MEDICAL SCHOOL

University of Regensburg	1982-84
University of Würzburg	1984-90
- clinical clerkships abroad: Glasgow (2 months), London (6 months), Vienna (1 months);	
- final year abroad: Madison/US (3 months), Krakow (3 months), Moscow (5 months)	

#### QUALIFICATIONS

1990	Graduation
1993	US Medical Licensing Examination, Part II
1994	US Medical Licensing Examination, Part I
1995	M.D. (University of Würzburg)
2000	Ph.D. (University of London)
2001	German Board Certification for Internal Medicine
2002	Habilitation

#### ADDITIONAL QUALIFICATION

1986	Russicum (university degree, Russian as a foreign language)
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#### PRESENT APPOINTMENT

Assistant Professor, Division of Hematology/Oncology Oregon Health & Science University, Portland OR	04/02 - present
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#### PREVIOUS APPOINTMENTS

Consultant, Department of Hematology University of Leipzig, Germany	2001 - 2002
Clinical Fellow, Department of Hematology University of Leipzig	1999 - 2001
Research Fellow, Department of Haematology Hammersmith Hospital, London, UK	1995 - 1999
Resident, Institute of Medical Oncology and Haematology Nürnberg General Hospital, Germany	1992 - 1995
Intern, Marienhospital Aachen, Germany	1990 - 1992

#### PROFESSIONAL MEMBERSHIPS

1998	German Society of Internal Medicine
------	-------------------------------------

1999 East German Study Group Hematology/Oncology  
 2000 Chronic Leukemia Working Party, EBMT  
 2000 American Society of Hematology

#### AWARDS/HONORS

1987 Study grant (Studienstiftung des Deutschen Volkes/German Scholarship Foundation)  
 1988 Study grant (Studienstiftung des Deutschen Volkes) to study medicine at the University of London  
 1989 12 months grant for final year at the 2. Moscow Medical Institute (DAAD/German Academic Exchange Service)  
 1995 1 year fellowship (Deutsche Krebshilfe) to study CML pathogenesis (Imperial College, London)  
 1996 1 year fellowship (Deutsche Krebshilfe) to study CML pathogenesis (Imperial College, London)  
 1997 Travel grant to attend the ASH meeting (British Society of Hematology)  
 1998 Travel grant to attend the Wilsede meeting (Leukemia Research Fund, UK)  
 1998 1 year fellowship (Dr Ernst und Anita Bauer Stitung, Germany) to continue Ph.D. at Imperial College  
 1998 1 year fellowship (Leuka2000, London) to continue Ph.D. at Imperial College  
 1999 Research Grant (Leukemia Research Fund): “*Characterisation of cell lines resistant to STI571*” (co-applicant with Dr Junia Melo) (\$100,000).  
 1999 Research Grant (University of Leipzig): “*Role of cyclin D2 in the pathogenesis of CML*” (\$10,000).  
 2000 Research Grant (Novartis): “*Prediction of response to STI571 in patients with myeloid blast crisis of CML*” (\$30,000).  
 2001 Research grant (Deutsche Krebshilfe): “*Charcaterisation of 2 Bcr-Abl-dependent genes*” (\$ 60,000).  
 2001 Research grant (IZKF Leipzig): “*Role of cyclin D2 in the pathogenesis of CML*” (\$75,000).  
 2001 Research grant (IZKF Leipzig): “*Gene array analysis to predict response to imatinib in CML patients*” (\$15,000).  
 2002 Research grant (Medical Research Foundation of Oregon): “*Clonality analysis of CML patients with complete cytogenetic response to imatinib*” (\$30,000).  
 2004 Fellow of the American Society of Hematology

#### OTHER SCIENTIFIC ACTIVITIES

##### ***Reviewer of Scientific Journals***

1998- Bone Marrow Transplantation  
 2000- Leukemia  
 2002- Oncogene  
 2002- Experimental Hematology  
 2002- Hematologica  
 2002- Blood  
 2002- Cancer Research  
 2003- The Lancet  
 2003- Cancer Cell  
 2003- Nature Genetics

##### ***Reviewer of Grants***

2002- Leukemia Research Fund

##### ***Membership in Scientific Committees***

2000- Molecular diagnostics (German competence network acute and chronic leukemias)  
 2001- Website “Chronische Myeloische Leukämie” (German)

### ***(Co-) Principal Investigator in Clinical Studies***

- “A phase II study to determine the efficacy and safety of STI571 in patients with chronic myeloid leukemia in myeloid blast crisis” (Novartis)
- “A phase II study to determine the efficacy and safety of STI571 in patients with chronic myeloid leukemia in accelerated phase” (Novartis)
- “A phase II study to determine the efficacy and safety of STI571 in patients with CML resistant or refractory to interferon-alpha” (Novartis)
- “Treatment of CML patients with imatinib + hydroxyurea- a phase I/II study” (East German Study Group Hematology/Oncology [OSHO])
- “Treatment of patients with newly diagnosed CML with pegylated interferon-alpha (pegasys<sup>®</sup>) – a phase I/II study” (OSHO)

### ***Co-Investigator in Clinical Studies***

- “A Phase I/II Dose-Finding Study to Determine the Safety, Tolerability, and Anti-Leukemic Effects of STI571 in Combination with Interferon-alpha in Patients with Chronic Myelogenous Leukemia in Chronic Phase” (National Institute of Health [NIH])
- “A Phase II Study to Determine the Anti-Leukemic Effects of STI571 in Combination with Ara-C in Patients with Chronic Myelogenous Leukemia in Chronic Phase” (NIH)
- “A Phase I/II Study to Determine the Safety, Tolerability, and Anti-Leukemic Effects of Trisenox (Arsenic Trioxide) in Combination with Gleevec (STI571) in Patients with Resistant Chronic Myelogenous Leukemia in Chronic Phase” (NIH)
- “Phase II Study Of AG-858 Plus Gleevec<sup>™</sup> In Patients With Chronic Myelogenous Leukemia (CML) In Chronic Phase Who Are Cytogenetically Positive After Treatment With Gleevec<sup>™</sup>”

## **CLINICAL EXPERIENCE**

### ***General Medicine***

Covered all areas of internal medicine including critical care. Experience in the setting of a small community hospital as well as tertiary referral center. Extensive experience in diagnostic procedures (echocardiography, sonography, gastroscopy).

### ***Hematology/Oncology***

Extensive experience in the diagnosis and management of patients with hematological malignancies and solid tumors. Several years of experience in high-dose therapy and autologous stem cell transplantation (mainly for lymphoma and germ cell tumors). Almost 3 years experience in allogeneic bone marrow transplantation, including reduced intensity conditioning.

### ***Hematological Diagnostics***

Standard morphology. Supervised molecular diagnostics in the Department of Hematology/Oncology at the University of Leipzig. Established or contributed to the establishment of qualitative and quantitative RT-PCR assays and Fluorescence in situ hybridization assays for molecular leukaemia diagnosis. Established data base and specimen repository.

## **RESEARCH EXPERIENCE**

### ***Clinical Research***

- Co - investigator in several Novartis-sponsored international multicenter trials that tested imatinib at various stages of CML and Ph-positive ALL.
- Co - PI in 2 multicenter trials of the East German Study Group Hematology/Oncology (OSHO) that test pegylated interferon and imatinib combined with hydrea
- Co-investigator in several NIH-sponsored multicenter trials that test imatinib in combination with conventional alpha-interferon, cytarabine and arsenic trioxide.



- Habilitation: “Hemmung der *Bcr-Abl*-Tyrosinkinase durch Imatinib, einen selektiven pharmakologischem Inhibitor: Präklinische und klinische Evaluation und Studien zur Pathogenese der chronischen myeloischen Leukämie (CML)”

### **Laboratory**

- MD thesis: “The value of bone marrow biopsies in clinical routine diagnostics: statistical and histological studies”.
- PhD thesis: “STI571, a novel tyrosine kinase inhibitor: pre-clinical evaluation and application to identify downstream targets of Bcr-Abl”
- Extensive experience in molecular biology techniques, including RT-PCR, Northern, Southern, Western blotting, expression cloning and protein expression, Fluorescence in-situ hybridization, cell culture, progenitor cell assays, FACS.

## **TEACHING EXPERIENCE**

### **Basic/Laboratory**

- Extensive experience in supervising research projects.
- Co-supervised PhD thesis of Dr Sara Vieira at Imperial College, London
- Supervised MD theses of Thomas Bumm and Uwe Reinhold at the University of Leipzig
- Co-supervised PhD thesis of Allie Grossmann (Oregon Health & Science University) (submitted June, 2003)
- Lecturer in Biochemistry (graduate course for Ph.D. students at Oregon Health & Science University)

### **Clinical**

- Supervised several final year medical students (Nürnberg General Hospital; University of Leipzig).
- Held lectures during in the main course “Internal Medicine” at Leipzig University
- Lecturer in Clinical Hematology (Graduate course for medical students at Oregon Health & Science University).

## **RESEARCH SUPPORT**

### **Ongoing Research Support**

No # (Deininger)	7/1/04-6/30/06
ASH Scholar Award	51%
	\$75,000

Epidemiology of clonal chromosomal abnormalities, analysis of clonality and assessment of DNA repair capacity in Ph-negative hematopoietic progenitor cells from CML patients with a complete cytogenetic response to imatinib  
The major goals of this project are to determine 1) the incidence, prognosis and associated risk factors for the development of karyotypic abnormalities in Ph-negative cells of CML patients treated with imatinib; 2) the incidence of clonal hematopoiesis in patients with a complete cytogenetic response to imatinib and if the Philadelphia-positive cell clone and the abnormal Ph-negative clone(s) originate from a common ancestral cell; and 3) the DNA repair capacity of Ph-negative progenitor cells from CML patient, with and without chromosomal abnormalities in Ph-negative cells, in the presence and absence of imatinib.

Role: PI

7393-06: Specialized Center of Research (Druker)	10/1/05 – 9/30/10
The Leukemia and Lymphoma Society	11%
	\$137,500

Molecularly Targeted Therapy for Leukemia (consortium with Dr. Charles Sawyers, UCLA, and Dr. John Kuriyan, UC Berkeley)

The major goals of Project 1 are to 1) fully characterize the biochemical and biological properties of the most commonly observed BCR-ABL mutants; and 2) define the mechanism of disease persistence in CML patients who are refractory to imatinib at the cytogenetic or molecular level, despite a complete hematologic remission.

Role: Co-PI, Project 1 – Evaluation of Mechanisms of Resistance to Imatinib in CML

1R01 HL082978-01 (Deininger)

9/20/05-8/31/09

NIH:NHLBI  
Biology of Imatinib-Resistant Mutants of BCR-ABL

23%  
\$225,000

The major goals of this project are to 1) determine differences in the transformation potencies of clinically detected BCR-ABL kinase domain (KD) mutants in comparison with wild type BCR-ABL; and 2) compare intrinsic kinase activity between wild type and mutant BCR-ABL and identify critical switches in signaling pathways that cause differences in transformation potency

Role: PI

### ***Completed Research Support***

Novartis Pharmaceuticals – Deininger (PI) 2000

Definition of mechanisms of resistance to imatinib in the myeloid  
blast crisis of CML

Role: PI

Interdisciplinary Center for Clinical Research – Deininger (PI) 2001

Prediction of cytogenetic response to imatinib using microarray technology

Role: PI

Interdisciplinary Center for Clinical Research – Deininger (PI) 2001-2002

Role of Cyclin D2 for the transformation of lymphoblasts by BCR-ABL

Role: PI

German Cancer Aid (Mildred Scheel Foundation) – Deininger (PI) 2001-2002

Molecular cloning and characterization of 2 BCR-ABL-regulated genes

Role: PI

(No #) – Deininger (PI) 6/1/03-5/31/04

5%

Medical Research Foundation of Oregon – Deininger (PI) \$30,000

Analysis of clonality in complete cytogenetic responders to imatinib

The major goals of this project were to 1) determine if the Bcr-Abl translocation is the only genetic lesion in the chronic phase of CML (i.e. is CML a two step genetic process); 2) determine if treatment with Gleevec will restore polyclonality; and 3) determine if Bcr-Abl positive cells and chromosomally abnormal Ph-negative cells arise from a common ancestor.

Role: PI

# CM030024: Hypothesis Development – Deininger (PI) 9/30/04-9/30/05

15%

Department of Defense Chronic Myelogenous Leukemia Research Program \$66,225

Elucidating the Mechanism of p27 Inactivation by the Bcr-Abl Tyrosine Kinase

The major goals of this project are 1) to determine whether Bcr-Abl regulates the activity of the SCFSKP complex and whether Skp2 is required for induction of leukemia in a murine model of CML and 2) to determine the cellular localization of p27, whether it is associated with phosphorylation of specific residues and whether it influences the degradation of the protein.

Role: PI

### **ADMINISTRATIVE EXPERIENCE**

Responsible for recruitment of scientific personnel (laboratory of Professor Brian Druker).

### **BIBLIOGRAPHY**

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3. Deininger, M.W. and J.M. Goldman, Chronic myeloid leukemia. *Curr Opin Hematol*, 1998. 5(4): p. 302-8.
4. Deininger, M.W., et al., Selective induction of leukemia-associated fusion genes by high-dose ionizing radiation. *Cancer Res*, 1998. 58(3): p. 421-5.
5. Gora-Tybor, J., et al., The susceptibility of Philadelphia chromosome positive cells to FAS-mediated apoptosis is not linked to the tyrosine kinase activity of BCR-ABL. *Br J Haematol*, 1998. 103(3): p. 716-20.
6. Deininger, M.W., J.M. Goldman, and J.V. Melo, The molecular biology of chronic myeloid leukemia. *Blood*, 2000. 96(10): p. 3343-56.
7. Deininger, M., et al., No evidence for persistence of BCR-ABL-positive cells in patients in molecular remission after conventional allogeneic transplantation for chronic myeloid leukemia. *Blood*, 2000. 96(2): p. 779-80.
8. Deininger, M.W., et al., BCR-ABL tyrosine kinase activity regulates the expression of multiple genes implicated in the pathogenesis of chronic myeloid leukemia. *Cancer Res*, 2000. 60(7): p. 2049-55.
9. Mahon, F.X., et al., Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. *Blood*, 2000. 96(3): p. 1070-9.
10. Marley, S.B., et al., The tyrosine kinase inhibitor STI571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. *Exp Hematol*, 2000. 28(5): p. 551-7.
11. Deininger, M.W., et al., Direct relation between BCR-ABL tyrosine kinase activity and cyclin D2 expression in lymphoblasts. *Cancer Res*, 2001. 61(21): p. 8005-13.
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14. Al-Ali, H.K., et al., CML with an e1a3 BCR-ABL fusion: rare, benign, and a potential diagnostic pitfall. *Blood*, 2002. 100(3): p. 1092-3.
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### **Book Chapters**

1. Deininger M. Grundlagen der Therapie mit Imatinib Mesilat in: Fischer T (ed.) Der Signalhemmer Imatinib Mesilat (STI571). Uni-Med Verlag, Bremen 2002 (German)
2. Deininger MW. Clinical applications in: Stein G and Pardee A (eds.). Cell cycle and growth control: biomolecular regulation and cancer. Wiley&Sons, N.Y., 2003 (*in press*)

***Invited Lectures at International Conferences***

- Identification of Bcr-Abl target genes using differential display (Biarritz, 1999)
- Mechanisms of defective cell cycle regulation in CML cells (Rapallo, 2000)
- Molecular pathogenesis of CML (Porto Allegre, 2001)
- Role of imatinib prior to allografting (Paris, 2001)
- Targeted therapy of CML with imatinib (Krakow, 2001)
- Imatinib prior to allogeneic transplantation (Madrid, 2002)
- Cytogenetics in CML patients on imatinib (Bordeaux, 2002)
- Role of imatinib for remission induction prior to allografting (Houston, 2002)
- Targeted therapy of CML with imatinib: a paradigm shift in cancer therapy? (Berlin, 2003)